IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Takehiko NOMURA et al.

Application No.: 10/522,877 Confirmation No.: 5247

Filed: February 2, 2005 Art Unit: 1651

For: Bacterial cell wall skeleton component preparation Examiner: S.R. Macauley

RULE 1.132 DECLARATION

MS AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

INTRODUCTORY COMMENTS

I, Takehiko Nomura, a citizen of Japan and residing at Osaka, Japan, say and declare as follows:

1. I received the degree Ph.D. from Kyoto University in Japan in 1998.

2. I have worked at Sumitomo Pharmaceuticals Research Center, now Dainippon Sumitomo Pharma from 1998 to 2008. I had been engaged in the study on pharmaceutical science and drug formulation until 2006.

3. I am an author or co-author of the following papers related to pharmaceutical science and cancer gene therapy:
Pharm Res. 1998 Jan;15(1):128-32;
J Control Release. 1998 Mar 31;52(3):239-52; and
Cancer Res. 1997 Jul 1;57(13):2681-6.

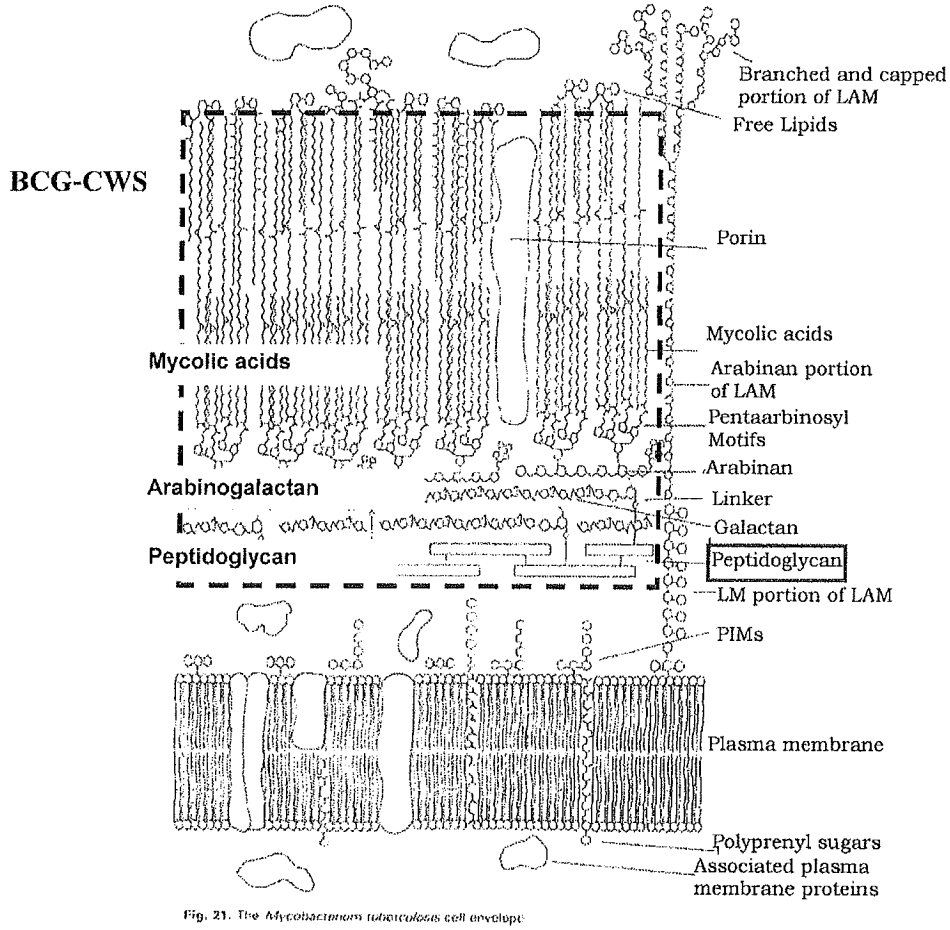
4. I am one of the inventors in U.S. Application, Serial Number 10/522,977 and I am very familiar with the subject matter thereof and have been researching the subject matter thereof since 1998.

5. I have performed, or supervised the performance of; the experiments described in the following paragraphs in support of patentability of the above-identified patent application.

6. Differences between the invention and the prior art:

(1) *Structural difference between muramyl dipeptide and BCG-CWS*

The "muramyl dipeptide" described in Van Nest reference has different physical properties from Bacillus-Calmette-Guerin Cell-Wall-Substance (BCG-CWS). Please see Fig. 1 below. "Muramyl dipeptide " is a part of BCG-CWS consisting of peptidoglycan.



Model structure of *M. Tuberculosis* by Brennan

Fig. 1: The structure of BCG-CWS.

(2) *Difficulty for preparing paste/emulsion comprising sub-micron droplet using BCG-CWS*

Van Nest discloses preparation of an emulsion comprising droplets of submicron size using muramyl dipeptide. Although Van Nest discloses that killed mycobacteria can be used as an immunostimulating agent, it fails to show any working examples wherein an emulsion using killed mycobaceria was actually prepared. Regarding the process for preparing an emulsion, Van Nest discloses that:

> The manner in which the droplet size of the invention is reached is not important to the practice of the present invention. One manner in

> which submicron oil droplets can be obtained is by use of a commercial emulsifiers, such as model number 11OY available from Microfluidics, Newton, Mass. Examples of other commercial emulsifiers include Gaulin Model 30CD (Gaulin, Inc., Everett, Mass.) and Rainnie Minilab Type 8.30 H (Miro Atomizer Food and Dairy, Inc., Hudson, Wis.). These emulsifiers operate by the principle of high shear forces developed by forcing fluids through small apertures under high pressure. When the model 11OY is operated at 5,000-30,000 psi, oil droplets having diameters of 100-750 nm are provided.

Van Nest, page 7, lines 15-21. Van Nest also indicates that "actual droplet size will vary with the particular detergent, oil, and immunostimulating agent (if any) and with the particular operating conditions selected." Van Nest, page 7, lines 25-26.

Van Nest discloses a working example to obtain a sub-micron droplet emulsion using muramyl dipeptide but not killed mycobacterium. That is, the strategy and expected particle size disclosed on page 7 lines 12-22 are the case when using muramyl dipeptide.

One of ordinary skill in the art could prepare emulsions containing uniform droplets of submicron size using muramyl dipeptide which is free from the problems of aggregation or adherence, by consulting Van Nest. However, when much larger material such as a CWS preparation from killed mycobacteria is used, preparing an emulsion containing uniform droplets of submicron size is extremely difficult and the ordinary artisan cannot obtain the sub-micron droplet emulsion as those recited in the instant claims even if the artisan consults Van Nest. Azuma is similarly silent about how to prepare such an emulsion. Thus, one skilled in the art, using a method known at the time of filing, would not have understood the importance of the particular conditions as described in this patent application needed for preparing the claimed BCG-CWS paste and therefore would not have been able to make the presently claimed BCG-CWS paste by reading Van Nest, Azuma, or both of them together.

(3) *Superior property of the paste of the invention: particle size distribution*

The paste of the present invention is obtained by a process for preparation which comprises the steps of (a) mixing the BCG-CWS and squalane in an organic solvent used as a dispersion-aiding solvent, wherein organic solvent is hexane or heptane which comprises 5 to 20 % (v/v) and (2) removing the organic solvent by distillation. In Example 10 of the instant Specification (page 53), a paste was prepared by the claimed method using 10% ethanol/90% heptane as the "dispersion-aiding solvent". The particle size distribution of thus obtained paste is shown in Table 12 (Specification, page 56).

In addition to the above, the originally filed Specification, Figures 1 and 2 represent the particle distribution of the paste when prepared using toluene alone (Specification, Fig. 1) or 10% ethanol/heptane (Specification, Fig. 2).

These results are illustrated together below:

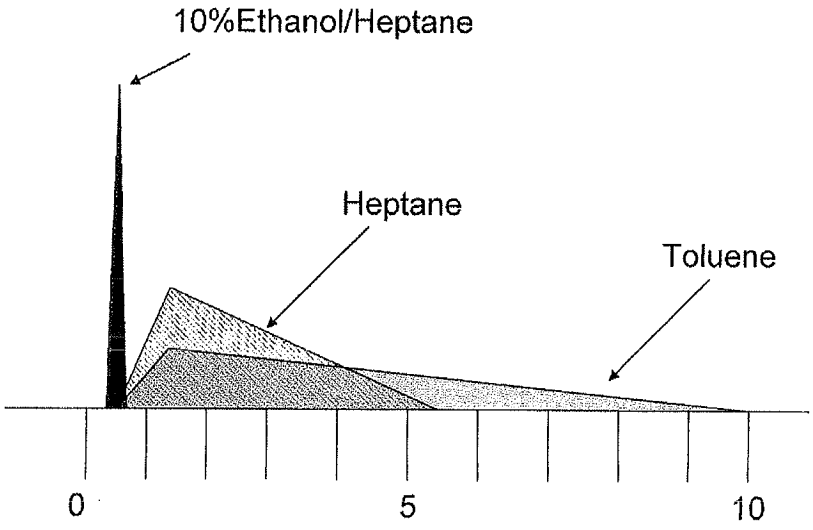


Figure 2: The X axis represents the particle size (μm).

The paste of the present invention prepared by using a mixture of 10% ethanol/heptane comprising droplets has a much sharper particle size distribution than those prepared using heptane or toluene alone. Van Nest does not provide any guide regarding how to prepare an emulsion of BCG-CWS comprising sub-micron droplets with such a sharp particle size distribution.

(4) *Superior property of the submicron paste of the invention: Significance of the viscosity*

The viscosity of the squalane is 0.236 poise, and that of a paste obtained by mixing squalane with BCG-CWS increases as the ratio of BCG-CWS to squalane is increases. (Specification, Example 3, page 46, line 4). As is evident from Example 4, a good oil-in water emulsion from the view points of (1) the efficiency of the assembly during emulsification; (2) particle size distribution immediately after emulsification; (3) particle size distribution before and after lyophilization; and (4) properties after resuspension of the lyophilized formulation can be prepared using a paste with a viscosity of 0.2-0.7 poise. (See Specification, page 46, line 16). In addition, Example 4 also evidences that the viscosity can be adjusted by controlling the ratio between BCG-CWS and squalane.

Further, a lyophilized formulation obtained by lyophilizing the emulsion of the invention prepared in Example 5 has unexpectedly superior properties of (1) the particle size distribution in the reconstituted formulation immediately after lyophilization shows a sharp peak with an average particle diameter of 2 to 3 μm, (2) there is no change in particle size distribution before and after lyophilization, (3) there is no adhesion of the formulation to the vial after resuspension of the lyophilized formulation nor decrease of the concentration of raw material in the formulation. (Specification, Example 5, page 48, line 20). Examples 15 and 16 also support the superior property of lyophilized formulation of the instant invention. (See Specification, page 59 line 15 to page 61, line 9).

Van Nest as well as Azuma are silent about this specific viscosity range and adjusting the viscosity of the emulsion by controlling the ratio between BCG-CWS and squalane.

9. Conclusion

For the reasons discussed above, one skilled in the art would not combine Azuma and Van Nest to achieve the present invention. Furthermore, the paste of the instant

application exhibits unexpected superior properties over Azuma and Van Nest. Therefore, the instant invention is not obvious over Azuma in view of Van Nest.

10. The undersigned declares further that all statement made herein of his own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statement may jeopardize the validity of above identified application or any patent issuing thereon.

Date: October 31, 2008 Takehiko Nomura

Dr. Takehiko Nomura, Ph.D.